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Microbiology of Fresh Comminuted Turkey Meat

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ABSTRACT

Standard plate counts, coliform plate and most probable number (MPN) counts, *Escherichia coli* plate and MPN counts, *Staphylococcus aureus* MPN counts, and fecal streptococcus counts were determined for 75 samples of fresh ground turkey meat purchased from retail markets in the San Francisco Bay Area. The presence of *Clostridium perfringens* was determined by both direct plate count and enrichment techniques. Salmonellae were isolated using selective enrichment procedures. Samples were screened for presence of enteroviruses. Aerobic gram-positive and gram-negative organisms were isolated and identified. *Clostridium perfringens* and *Salmonella* sp. were isolated from 52% and 28% of the samples, respectively. The mean standard plate count was 84,000,000 per gram. The mean count for *E. coli determined by the MPN method was 19 per gram.* Fecal streptococci were isolated from 95% of the samples with a mean count of 18,000 per gram. *Staphylococcus aureus* was isolated from 80% of samples analyzed with a mean count of 34 per gram.

The amount of poultry consumed per capita in this country has steadily increased since the 1930's. This increase can be attributed to improvement in poultry production through breeding, nutrition, and disease control as well as processing procedures within the industry.

The economic need to use as much of the fowl as possible and improvements in processing techniques have resulted in marketing of several types of cut-up and processed turkey products. Further processed products, prepared from deboned turkey meat range from turkey rolls to ground or comminuted turkey meat.

It is estimated that nearly 50% of the beef consumed in this country is in the comminuted form. Much of this is consumed in a semicooked condition (*13*). Due to the popularity and economical aspects of meals prepared with comminuted beef, the consumer is being encouraged to try other comminuted meat products such as turkey. Turkey, in the comminuted form, is advertised as being low in cost, fat, and calories, yet high in some essential daily dietary components such as protein, niacin, and riboflavin (*29*).

The literature contains reports of two studies dealing with the microbiology of poultry meat (*27, 30*). The meat used in these studies was not purchased at the retail level but was obtained from either university or commercial poultry processing plants (*27, 30*).

Comminuted turkey, a further-processed product prepared from the dark meat of the fowl, is deboned by hand or by machine depending on the wholesale supplier (*23, 36*). Fresh or frozen turkey is obtained as a coarse grind and is generally reground at the retail level before being offered for sale in either prepackaged units or by open-try selection. Comminution of a product greatly increases the surface area, distributes the bacteria throughout, and oxygenates the product. Comminuted products, if mishandled, have long been recognized for their potential to support and promote rapid growth of microorganisms.

Several reports have implicated poultry and poultry products as important sources of organisms belonging to the tribe *Salmonellae* (*9, 11, 12, 19, 20, 37*). On several occasions, meat from turkeys has been implicated in outbreaks of salmonellosis (*4, 35*). Bryan et al. (*12*) demonstrated that dissemination of *Salmonellae* by turkeys begins on the farm. Their study indicated that during processing procedures, equipment and meat become contaminated with the organisms carried by the turkeys in their feces and on their feet and feathers.

Surveys of dressed poultry from processing plants and retail stores have revealed *Salmonellae* contamination present in 1 to 50% of the samples tested (*18, 32, 39*). Since comminuted turkey is a new product, its level of bacterial contamination is not reflected in retail market surveys conducted before this study.

Consumer awareness of potential public health problems and consumer generated pressures for protective legislation have resulted in the May 1973 enactment of Oregon's microbiological standards for meat products. The adopted standards for ground and whole cuts of red meats established a limit of 5 million organisms per gram for the total aerobic plate count and a limit for *Escherichia coli* of 50 per gram determined by the most probable number (MPN) method (*13*). Canadian officials have proposed microbial standards for ground meat items. This proposal establishes a limit for the standard plate count of 10 million per gram while the limit of 50 per gram was set for *E. coli* (*3*). With standards such as these already in existence for red meat items, consumer pressure may influence legislative

bodies to enact appropriate standards for poultry products. Therefore, studies of the microflora of comminuted poultry products purchased at the retail level are necessary before adoption of standards for these items.

MATERIALS AND METHODS

Samples

Refrigerated samples, in approximately 1 lb lots, were procurred at various retail markets in the San Francisco Bay Area. They were transported to the laboratory in a Freez Safe Styrofoam Case (GloBrite Foam Plastics Co., Chicago, IL) using Blue-Ice cold storage usits (Divajex Co., Santa Ana, CA) to provide a refrigerated atmosphere of approximately 8 C. Samples for bacteriological analysis were either analyzed immediately or after holding at 4 C for not longer than 48 h. Samples virological analysis were placed in sterile 50-ml centrifuge tubes and stored at -80 C until analysis was initiated.

Bacteriological analyses

Various media were used to indicate the numbers and types of organisms present. Aerobic plate counts were determined using Standard Methods Agar and incubation at 32 C for 72 h.

Estimates of coliforms were made by the plate count procedure with Violet Red Bile (VRB) agar as well as by the Most Probable Number (MPN) technique as described in *Standard Methods for the Examination of Dairy Products* (*1*).

Selenite and Tetrathionate broths, for detection of salmonellae, were incubated at 37 C for 24 h. The broths were then streaked to plates of *Salmonella-Shigella*, Bismuth Sulfite, and Brilliant Green Sulfadiazine agars and incubated for 24 h at 37 C. Colonies exhibiting positive reactions on these media were verified biochemically and serologically following procedures outlined in *Identification of Enterobacteriaceae* (*17*).

Determination of the *Staphylococcus aureus* MPN was done using the method outlined by the Association of Official Analytical Chemists (AOAC), with the substitution of Tellurite Polymyxin Egg Yolk agar for Vogel and Johnson agar. The AOAC procedure was used for determination of coagulase production (*7*).

Procedures from the *Bacteriological Analytical Manual for Foods* (*5*) were used for enumeration of fecal streptococci. In addition to these procedures, colonies from KF Streptococcal agar were transferred to Ethyl Violet Azide broth for confirmation. After incubation at 37 C for 48 h, colonies were considered confirmed if a yellow color developed and sediment was observed in the tube.

The presence of *Clostridium perfringens* was determined by aseptically removing five aliquots of turkey meat, 1 to 2 g each, and placing these in tubes containing Fluid Thioglycollate medium. Tubes were incubated for approximately 6-8 h in water bath set at 46 C. After this incubation period, cultures exhibiting profuse gas production were transferred to Cooked Meat Medium and incubated overnight at 37 C. Fluid Thioglycollate tubes showing little or no gas production after the 46 C incubation period were also incubated overnight at 37 C. If, after overnight incubation, gas was observed, the culture was transferred to Cooked Meat Medium. Tubes not exhibiting evidence of gas production were discarded. Presumptive *C. perfringens* isolates were purified and verified using Sulfite-Polymyxin B-Sulfadiazine agar (SPS), Liver Veal Egg Yolk agar, as well as gram stain, Iron Milk, and Nitrite Motility medium. The serum neutralization mouse assay technique was used for typing isolated strains (*16*).

Isolation and identification of aerobic bacteria present in each turkey sample were accomplished using the following procedures: Gram-negative organisms were isolated from the remaining sample by inoculating 10 ml double strength GN broth with 10 ml of the 10^{-1} dilution originally prepared for the total plate count procedure. After 24 h of incubation at 35 C, the sample was inoculated onto *Salmonella-Shigella*, Bismuth Sulfite, Brilliant Green Sulfadiazine, Hektoen Enteric, MacConkey's, and Eosin-Methyline Blue (EMB) agar plates. Following incubation at 37 C for 24 h, representative colony types were subcultured to EMB agar to assure purity. *Identification of gram-negative isolates was made using the* API 20E *Enterobacteriaceae* system (Analytab Products, Inc.). The Analytical Profile Index was used to assign species designations to isolates identified by this procedure.

Gram-positive organisms were isolated from the sample by inoculating 10 ml double strength Trypticase Soy Broth (TSB) containing 4.0 mg potassium tellurite/ml with 10 ml of the original 10^{-1} food slurry. In addition, 1 ml of the original food slurry was inoculated into 10 ml of TSB. Tubes were incubated overnight at 35 C before being inoculated onto Mueller-Hinton agar plates containing 5% defibrinated sheep blood. Representative colony types were subcultured to blood agar plates to assure culture purity. After overnight incubation at 37 C, isolates were separated into two categories on the basis of catalase demonstration in 3% hydrogen peroxide.

Catalase positive organisms exhibiting coccal morphology were examined for their oxidative-fermentative capabilities in O-F Basal medium containing 1% glucose. In addition, mannitol utilization and nitrate reduction were determined. Isolates utilizing mannitol and exhibiting fermentative ability were tested for coagulase production. Rod-shaped catalase positive organisms were inoculated into a primary battery of media including purple broth base with 1% glucose, Simmon's Citrate agar slant, Indole-Nitrite-Motility medium, and a Trypticase Soy agar plate.

Streptococci were speciated using a multipoint inoculator (Melrose Machine Shop, Woodlyn, PA) and inoculating isolates onto the following agar media: blood, 40% bile, glucose, 6.5% sodium chloride, bile-esculin-azide, gelatin, starch, arabinose, glycerol, lactose, mannitol, raffinose, sorbitol, melibiose, and melezitose. Isolates were also examined for their ability to grow at 10 and 45 C.

Virological analysis

Samples of comminuted turkey, approximately 4 g, were put into 40 ml of skim milk and centrifuged for 20 min at 2,000 rpm. Supernatant fluid was treated with 100 μg of gentamicin and 5 μg of fungizone per ml. A 0.2-ml inoculum of supernatant fluid was transferred into each of two tubes of the following cell lines: HEK, VMK, PRMK, and WI-38. Cell cultures were incubated at 33 C in L-15 maintenance medium and read twice weekly on days 2 and 4 for cytopathogenic effect. On the seventh day, a blind passage was made and allowed to incubate 11 days before regarding the sample as negative.

RESULTS

The distribution of standard plate counts can be seen

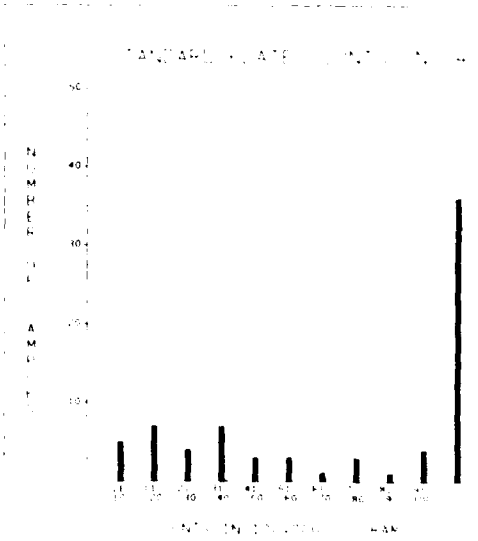

Figure 1. *Distribution of plate counts when samples contained up to 10,000,000 bacteria/g.*

in Fig. 1. Due to a laboratory accident, standard plate counts were recorded for only 74 of 75 samples. The counts ranged from 7.4×10^4 to 3.0×10^9 per gram with a mean count of 8.4×10^7 per gram. Thirty-five percent of the samples had aerobic plate counts of 5.0×10^6 or less. Fifty-one percent of the samples tested had aerobic plate counts of 1.0×10^7 per gram or less. Figure 2 shows the distribution of the 36 samples which had standard plate

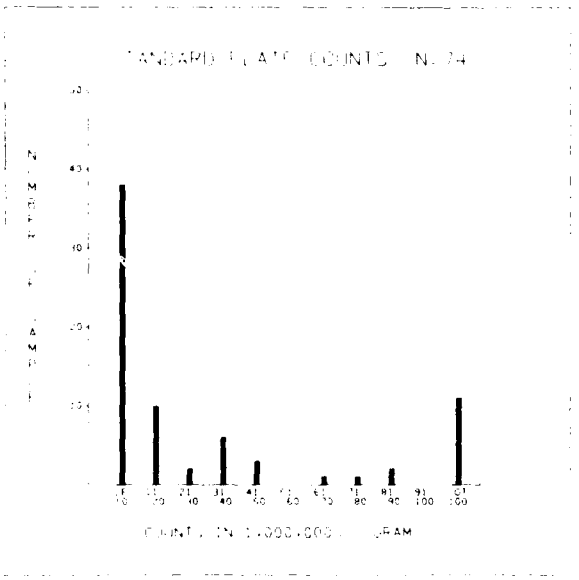

Figure 2. *Distribution of plate counts when samples contained more than 10,000,000 bacteria/g.*

counts greater than 1×10^7 per gram. Thirty-one percent of these samples had counts greater than 1×10^9 per gram.

Results of coliform determinations made by both the plate count and the MPN methods, can be seen in Fig. 3 and 4, respectively. Analysis by the coliform plate count

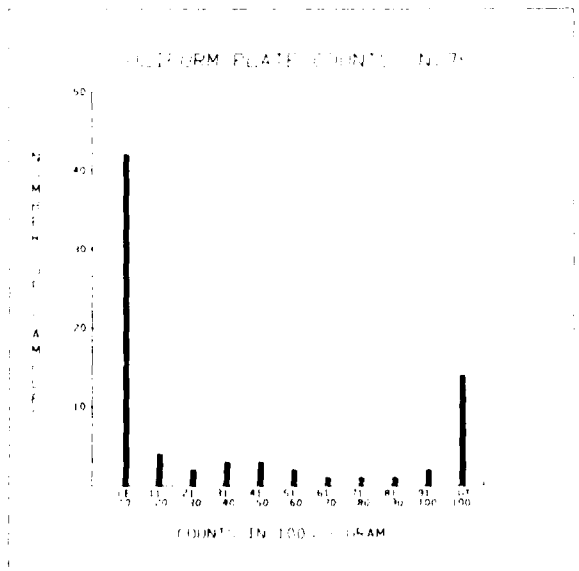

Figure 3. *Distribution of coliform plate counts.*

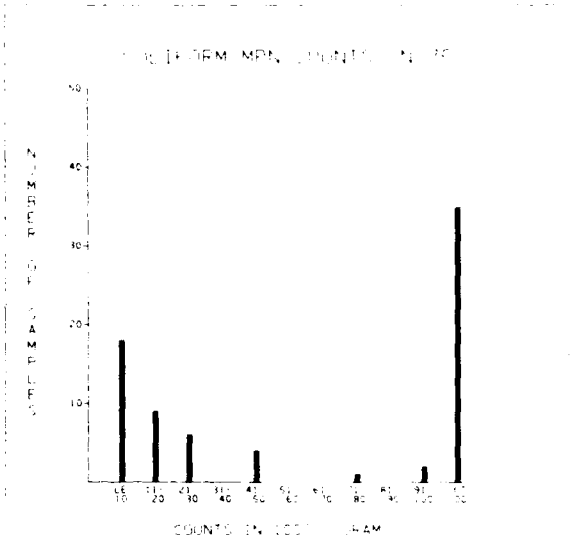

Figure 4. *Distribution of coliform MPN counts.*

method yielded counts ranging from 5.0×10^0 to 4.8×10^5 with a mean of 2.0×10^4 per gram. Using this technique, coliforms were not detected in 12% of the samples. Using the MPN technique, counts for the 75 samples ranged from 2.3×10^1 to 1.1×10^7 with a mean count of 6.3×10^4 per gram. Coliforms were detected in all but one sample with this testing procedure.

Use of the MPN technique facilitated detection of *E. coli* in 41% of the samples. Confirmed *E. coli* counts ranged from 3.6×10^0 to 7.5×10^2 with a mean count of 1.9×10^1 per gram. By the plate count procedure, *E. coli* was detected in only 8% of the samples with counts ranging from 5.0×10^0 to 6.0×10^3 with a mean of 8.7×10^1 per gram.

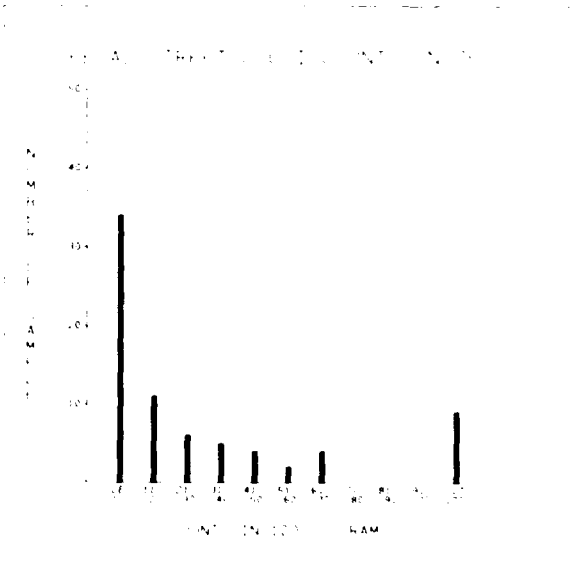

Figure 5. *Distribution of fecal streptococcus counts.*

Figure 5 shows the distribution of counts for fecal streptococci detected in 95% of the samples. Counts from positive samples ranged from 4.0×10^1 to 3.0×10^5 per gram. The mean count for the 75 samples was 1.8×10^4 per gram.

Staphylococcus aureus was detected in 69% of the samples tested by the MPN techique. Distribution of counts for these samples can be seen in Fig. 6. Counts ranged from 3.6×10^0 to 1.1×10^3 with a mean of 3.4×10^1 per gram.

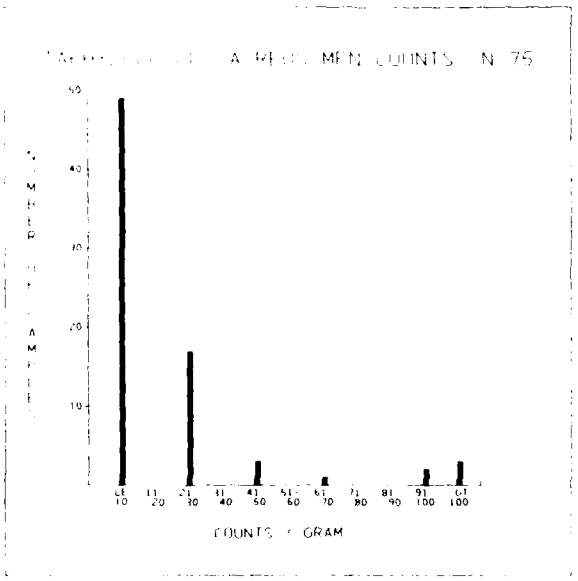

Figure 6. *Distribution of* Staphylococcus aureus *MPN counts.*

Twenty-one samples yielded isolates belonging to the genus *Salmonella.* Most of these isolates (52%) were *Salmonella enteriditis* bioserotype *pullorum.* Of the salmonellae isolated, 33% were identified as *Salmonella arizonae* while *Salmonella enteriditis* serotype *Saint Paul, Salmonella enteriditis* bioserotype *Miami* and *Salmonella enteriditis* serotype *Derby* each respresnted 5% of the salmonellae isolations.

The enrichment procedures used in this study yielded a variety of isolates. Table 1 lists the number of isolations and the percentage of samples represented for each of the aerobic organisms as well as *C. perfringens.* The most frequently isolated organisms were *Citrobacter freundii, Hafnia alvei* (*Enterobacter hafniae*), and *Escherichia coli* isolated from 87, 83, and 81% of the samples, respectively.

The enterovirus isolation procedure used in this study yielded no isolates.

TABLE 1. *Bacterial flora isolated from fresh comminuted turkey meat*

Organism	Number of isolations	% of samples
Gram-positive Isolates		
Bacillus polymyxa	1	1
Bacillus sp.	2	3
Clostridium perfringens	39	52
Corynebacterium sp.	1	1
Micrococcus sp.	26	35
Staphylococcus aureus	60	80
Staphylococcus epidermidis	50	67
Streptococcus acidominimus	2	3
Streptococcus agalactiae	1	1
Streptococcus anginosus	1	1
Streptococcus bovis	2	3
Streptococcus cremoris	2	3
Streptococcus dysgalactiae	1	1
Streptococcus equi	1	1
Streptococcus equisimilis	2	3
Streptococcus faecalis	35	47
Streptococcus faecalis var. *liquefaciens*	55	73
Streptococcus faecium var. *Casseliflavus*	2	3
Streptococcus faecium var. *durans*	1	1
Streptococcus lactis	20	27
Streptococcus *sp.*	*3*	*4*
Gram-negative Isolates		
Acinetobacter calcoaceticus var. *anitratum*	11	15
Acinetobacter calcoaceticus var. *lwoffi*	1	1
Alcaligenes sp. (*Achromobacter* sp.)	6	8
Citrobacter diversus	4	5
Citrobacter freundii	65	87
Enterobacter aerogenes	6	8
Enterobacter agglomerans	11	15
Enterobacter cloacae	52	69
Enterobacter liquefaciens (*Serratia liquefaciens*)	19	25
Escherichia coli	61	81
Flavobacterium sp.	1	1
Hafnia alvei (*Enterobacter hafniae*)	62	83
Klebsiella pneumoniae	48	64
Proteus inconstans (*Providencia alcalifaciens*)	3	4
Proteus mirabilis	11	15
Proteus morganii	12	16
Proteus rettgeri	1	1
Proteus vulgaris	4	5
Pseudomonas aeruginosa	16	21
Pseudomonas fluorescens	5	7
Pseudomonas fluorescens grp.	5	7
Pseudomonas maltophilia	2	3
Salmonella arizonae (*Arizona hinshawii*)	7	9
Salmonella enteriditis bioser. *Miami*	1	1
Salmonella enteriditis bioser. *pullorum*	11	15
Salmonella enteriditis ser. *Derby*	1	1
Salmonella enteriditis ser. *Saint Paul*	1	1
Serratia marcescens	5	7
Yersinia enterocolitica	3	4

DISCUSSION

The mean standard plate count reported in this study is considerably higher than counts for other comminuted meat products which have been reported by several investigators (*22, 24, 33*). Maxey et al. (*27*), reported counts ranging from 1×10^5 through 1×10^6 per gram of comminuted poultry meat including chicken. Ostovar et al. (*30*), compared immediately processed and delayed processed deboned poultry meat samples. In their study, standard plate counts of 3.3×10^5 per gram for the immediate process product and 7.1×10^5 per gram for

the product produced by the delayed processing procedure were reported.

The high standard plate counts reported in this study are most likely due to the additional grinding and handling as well as the temperature and length of refrigerated storage of the product before purchase at the retail level. The use of skin in the comminuted product probably contributes to high bacterial counts observed. Walker and Ayres (*37*) reported recovery of 4,700 organisms/cm^2 from the skin of live turkeys. They found that following processing, the counts increased to 44,000/cm^2 of skin surface. Current manufacturing procedures include use of 8-14% skin in the preparation of the product (*23*). This procedure provides the turkey meat with an inoculum of spoilage organisms as well as organisms of public health significance.

It is noteworthy that if the bacterial standards for ground beef items adopted in Oregon (*13*) and those proposed in Canada (*3*) were applied to the samples in this study, only 35% of the samples tested would comply with the Oregon standard for total bacterial count while 51% of the samples would comply with the proposed Canadian standards.

Using the MPN technique, *E. coli* was isolated from 41% of the samples while the plate method resulted in only eight isolates. This is evidence for the lack of sensitivity inherent with the plate count procedure for enumeration of this organism.

The fact that most streptococci isolated were identified as *Streptococcus faecalis* (25%) or its variety *liquefaciens* (40%) is in accord with the findings of Wilkerson et al. (*40*). They reported *Streptococcus faecium* to be the most prevalent enterococcus recovered from turkeys before processing while following processing, *S. faecalis* was found to be the most frequent enterococcus isolated.

In discussion of the importance of enteric bacilli in foods, it is necessary to consider the history, method of processing, and treatment the product receives before consumption. Since techniques for aseptic removal of the intestinal tract have not yet been developed, enteric bacilli will be found on the surfaces of red meat and poultry products in abattoirs and processing plants. Cooked red meat products with their natural enteric flora have rarely been associated with illness in man (*15*). Poultry and poultry products, on the other hand, are the most frequently incriminated sources in foodborne outbreaks of *Salmonella* infections (*31*).

Good processing procedures may result in low numbers of contaminants during the slaughtering and chilling operations in poultry processing. However, at temperatures above freezing, surface growth of aerobic, psychrotrophic organisms will occur. Comminution of the poultry meat greatly increases the surface area and creates a new environment for the organisms by distributing them throughout the product. Additionally, comminution increases the chances of contamination by providing further opportunity for product contact with surfaces of counters, machinery, knives, and hands of workers.

As a rule, properly cooked meats are not associated with gastro-intestinal illness in man. The consumer is being encouraged to try comminuted turkey as a substitute in recipes usually calling for hamburger (*2, 29*). Considering the bacterial load as reported in this study, the consumer should also be advised not to consume comminuted turkey in the rare or raw state as is often done with hamburger.

Comminuted turkey may also serve as the vehicle carrying organisms directly from the animal to a food-preparation establishment or indirectly, via the hands of the kitchen worker, to another food product which may be subsequently consumed in an uncooked state.

Indirect aspects of transmission become important when one considers the percentage of samples (see Table 1) yielding isolates of *Enterobacter* sp., *Klebsiella* sp., *S. aureus, C. perfringens,* and *Salmonella* sp.

The *Klebsiella-Enterobacter-Serratia* group of organisms, implicated as the predominant group of *Enterobacteriaceae* on spoiled poultry (*14*), represented 52% of the *Enterobacteriaceae* isolates from this study. *Klebsiella pneumoniae,* isolated from 64% of the samples studied, can be commonly associated with human respiratory and genitorinary infections (*8*). *Enterobacter cloacae,* found in 69% of the samples, has been associated with bacteremias as well as urinary tract infections (*8*).

Most *Salmonella* isolates in this study were *S. enteroditis* bioser. *pullorum,* an organism characterized as having a low level of infectivity for man (*20, 21*). *S. enteriditis* ser. *St. Paul, S. enteriditis* bioser. *Miami, S. enteriditis* ser. *Derby* and *S. arizonae* isolated in this study are all readily capable of causing human infection (*4, 21*).

Coagulase-positive staphylococci, recovered from 80% of the samples, can suggest the existence of carrier states among the birds and the personnel processing the product. Since *S. aureus* is known to be a weak competitor for growth in the presence of large numbers of other bacteria, it is doubtful that this product would be involved in outbreaks of staphylococcal intoxication. However, if sodium chloride were added and the product subsequently mishandled through lack of refrigeration, the rapid rate of cell replication might result in a product containing large numbers of enterotoxin producing staphylococci. This product would remain harmful even after cooking as some of the enterotoxins have been characterized as heat stable (*10*).

Clostridia are commonly found in our environment and their wide distribution in poultry processing plants has been demonstrated (*26, 28*). These organisms enter the slaughter plant on the feet, feathers, intestinal contents, and dirt associated with live birds. After scalding and plucking, however, few of these organisms can be recovered from the fowl surface (*28*). Lillard (*25*) has demonstrated that submersion of poultry in scald tanks can result in internal contamination of carcasses. She found that internal carcass contamination is not

washed off and may not be as easily destroyed by cooking as are surface contaminants.

By using enrichment techniques and five, 1 to 2-g portions of meat, *C. perfringens* was recovered from 52% of the samples. By the direct plating techniques, plating a 0.2-g sample with SPS agar, *C. perfringens* was not detected in any of the samples. Lack of isolation by this method may be due to low levels of contamination in conjunction with a small sample size, cell injury sustained during exposure to high and low temperatures, and increased susceptibility to Sulfadiazine and Polymyxin B. Of the *C. perfringens* isolates obtained in this study, 36 of 36 strains typed were identified as type A by the serum neutralization mouse assay method. The remaining three strains were lost through a laboratory accident, before typing.

Several bacterial organisms have, from time to time, been proposed for use as indicators of the hygienic quality in food products. As can be seen in the literature, none of these has met all criteria established for indicator organisms. With this in mind and knowing that enteroviruses have been isolated from samples of comminuted beef (*34*), it was decided to screen turkey samples for the presence of enteroviruses as possible indicators of contamination. Tissue culture under fluid media was the assay system chosen. This culture system is sensitive to a wide variety of viruses, observable for a long period, and may be carried through several passages. Failure to detect virus present in any of the samples may have been due to either the small sample size chosen for analysis or the absence of virus in the product.

Poultry products with high bacterial counts have been termed undesirable as far as keeping quality, public health aspects, and general esthetic principles are concerned (*3, 26*). Comminuted turkey meat purchased fresh at the retail level is a product with a high degree of bacterial contamination, limited keeping quality, and the ability to convey salmonellae and other enteric bacilli, staphylococci, and clostridia to the surfaces of equipment and the hands of kitchen workers in homes, hospitals, and factories.

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Standard plate counts, coliform plate and most probable number (MPN) counts, Escherichia coli plate and MPN counts, Staphylococcus aureus MPN counts, and fecal streptococcus counts were determined for 75 samples of fresh ground turkey meat purchased from retail markets in the San Francisco Bay Area. The presence of Clostridium perfringens was determined by both direct plate count and enrichment techniques. Salmonellae were isolated using selective enrichment procedures. Samples were screened for presence of enteroviruses. Aerobic gram-positive and gram-negative organisms were isolated and identified. Clostridium

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perfringens and Salmonella sp. were isolated from 52% and 28% of the samples, respectively. The mean standard plate count was 84,000,000 per gram. The mean count for E. coli determined by the MPN method was 19 per gram. Fecal streptococci were isolated from 95% of the samples with a mean count of 18,000 per gram. Staphylococcus aureus was isolated from 80% of samples analyzed with a mean count of 34 per gram.